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Biochemical changes in dehydrogenase, hydroxylase and tyrosinase of a permethrin-resistant strain of housefly larvae, *Musca domestica* L.

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Abstract

In the present study, a permethrin-resistant strain (ALHF) of housefly was used to understand some enzymic changes in normal biosynthetic pathways after insecticide selection. Aflatoxin B_1 (AFB_1) as a natural substrate was used to verify the changes on the level of cytochrome P450-dependent monooxygenases and oxido-reductase activities in the ALHF strain compared to an insecticide-susceptible strain, aabys. ALHF yielded three major biotransformation products: aflatoxin B_{2a} (AFB_{2a}), aflatoxin M_1 (AFM_1), and aflatoxicol (AFL) by larvae. These principal products were also found in aabys. AFL production rate of ALHF larvae was 5-fold lower than that of aabys. Differences between ALHF larvae and aabys in AFM_1 production were found. ALHF did not differ significantly from aabys in AFB_{2a} production. The levels of 17α- and β-hydroxysteroid dehydrogenase (17α- and β-HSD) were also determined to elucidate which type of dehydrogenase activities could be changed. The cytosolic fraction of ALHF larvae yielded about 2-fold higher 17α-estradiol than that of aabys larvae. In contrast, the microsomal fraction of ALHF larvae produced about 2-fold lower amount of 17α-estradiol than that of aabys larvae. The production rate of microsomal fraction of 17β-estradiol ALHF larvae yielded 3-fold lower than that of aabys larvae. Inhibition studies on 17α-HSD and 17β-HSD activities by pyrethroid insecticides showed that there was no inhibition by pyrethroids on the enzyme activity. Therefore, there seems to be no changes on the enzyme structures. Changes on enzyme expression may occur in ALHF larvae in relation to 17α- or β-HSD. To assess biochemical changes of the cuticle formation phenylalanine 4-hydroxylase and tyrosinase activities were determined. The production rate of tyrosine from phenylalanine in ALHF was about 2-fold higher for larvae than that in aabys. L-(dihydroxylphenyl)alanine (DOPA) content was determined in larvae and ALHF possessed 1.6-fold larger amounts of DOPA than aabys. Tyrosinase activity of ALHF larval preparations showed 1.6-fold higher than aabys. In summary, many enzymic changes were found in ALHF strain compared to aabys strain and these changes may be resulted from the permethrin selection.

Keywords: Housefly; Permethrin resistance; Aflatoxin B_1 dehydrogenase; 17β-Hydroxysteroid dehydrogenase; Phenylalanine-4-hydroxylase; Tyrosinase

1. Introduction

Many chemicals are used as pesticides and therapeutic agents not only to improve human health but also to reduce food shortages throughout the world. However, their continued use often results in resistance to these chemicals in the target organisms which can develop behavioral avoidance or physiological and biochemical mechanisms to allow them to survive under levels of the chemicals that kill susceptible individuals. Some organisms have a natural tolerance to chemical treatments that will kill other species. Resistance develops through a process of selection for rare individuals in a population with a gene which confers an advantage to

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the individual in the presence of a toxic chemical (Bourguet et al., 2000).

The resistance mechanisms available to insects may be divided into three general categories. The first category includes modified behavioral mechanisms that reduce the exposure of an insect to toxic compounds (Sparks et al., 1989). In the second category are physiological mechanisms including altered penetration and excretion of insecticides by insects (Little et al., 1989; Clark et al., 1995). The third category relies on biochemical mechanisms such as the insensitivity of target sites to insecticides (Zhao et al., 2000; Walsh et al., 2001; Weill et al., 2002) and enhanced detoxification by several metabolizing enzymes (Lee et al., 2000; Lee and Lees, 2001).

The housefly, *Musca domestica* L., is one of the most widely distributed organisms and occupies a basic position in the food chain. Pyrethroid insecticides are frequently used to control housefly, *Musca domestica* L., which is a serious public health insect pest. Pyrethroids attack sodium channels in the insect nervous system. However, the housefly has shown a remarkable ability to be resistant to pyrethroids and now many housefly species possess cross-resistance to new pyrethriods and new classes of insecticides (Liu and Scott, 1997). Knock-down resistance (*kdr*) can be related to changes in the sodium channels in the nervous system due to pyrethroid toxicity and housefly has developed *kdr* resistance type to pyrethroids (Williamson et al., 1993; Castella et al., 1997; Smith et al., 1997). This resistance may be related to a variation in the number of sodium channels (Bull and Pryor, 1990) or to altered-binding capacity of the channels for pyrethroids (Pepper and Osborne, 1993). Molecular biological studies support a relationship between an alteration of the binding site of pyrethroid on the sodium channel and the *kdr* resistance trait (Williamson et al., 1994). On the other hand, Scott and Georghiou (1986) have demonstrated that higher levels of cytochrome P450, cytochrome b_5 and NADPH-cytochrome *c* reductase activities were correlated with increased monooxygenase activities that was shown by synergism with piperonyl butoxide (PBO) to be the major resistance mechanism to permethrin in the resistant LPR strain of housefly. Liu and Yue (2000) have demonstrated an Alabaman housefly strain (ALHF) collected in 1998 from a poultry farm after a failure of control with permethrin, a pyrethroid insecticide, was 1800-fold resistant to permethrin after further selection and the resistance was related to increased levels of cytochrome P450-dependent monooxygenases and esterases. Therefore, enhanced metabolism of pyrethroids by detoxifying enzymes is one of resistance mechanisms developed in housefly.

However, cytochrome P450-dependent monooxygenases systems are implicated in many biosynthetic and metabolic pathways in insects. For example, cytochrome P450-dependent monooxygenases catalyze hydroxylation reaction of phenylalanine at 4-carbon position to produce tyrosine that is a key amino acid in catecholamine biosynthesis and cuticle sclerosis (Li et al., 1994; Ui-Tei et al., 1994; Lee and Campbell, 2000). For these reasons, a biosynthetic pathway in a pyrethroid-resistant strain of housefly can be changed due to the modified detoxifying enzymes. However, there is little information on physiological and biochemical changes in biosynthetic or metabolic pathways in insecticide-resistant insects after selection to insecticides. Here, we report enzymic changes such as 17α- and β-hydroxysteroid dehydrogenase (17α- and β-HSD), AFB_1 dehydrogenase, phenylalanine-4-hydroxylase, and tyrosinase in ALHF strain of housefly after selection to permethrin compared to an insecticide-susceptible strain. The natural substrates used in this study are shown in Fig. 1.

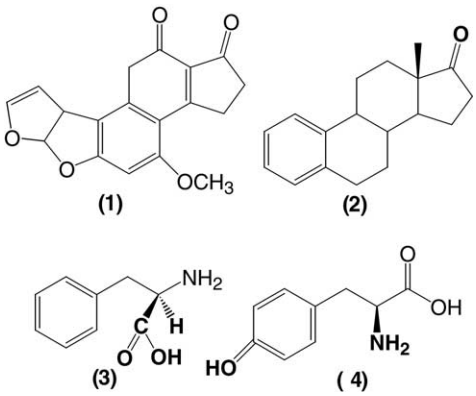

Fig. 1. Structures of natural substrates used in this study: (1) aflatoxin B_1; (2) estrone; (3) phenylalanine; (4) tyrosine.

2. Materials and methods

2.1. Housefly strains

ALHF, a strain collected from a poultry farm, near Grant, Marshall County, AL, in 1998 after a control failure with the use of permethrin. An insecticide-susceptible strain, aabys, obtained from Prof. J.G. Scott, Cornell University, Ithaca, NY, and flies were reared as described previously (Liu and Yue, 2000).

2.2. Insecticides and chemicals

Bifenthrin (96%), cypermethrin (92%), deltamethrin (99.9%), and permethrin (95.3%) were supplied by FMC (Princeton, NJ). AFB_1, AFM_1, AFB_{2a}, AFG1, AFG_2, L-phenylalanine, L-dihydroxyphenylalanaine (L-DOPA), estrone, 17α-estradiol, and 17β-estradiol were purchased from Sigma (St. Louis, MO). Aflatoxin B_1-8,9-epoxide (AFBO) was synthesized as previously described (Iyer and Harris, 1993). Aflatoxin epoxide-glutathione conjugate was biosynthesized according to the protocols of Raney et al. (1992). All synthesized chemicals were pure as determined by HPLC and MS. L-Tyrosine was purchased from Eastman Kodak Co. (Rochester, NY). All purchased chemicals were of the highest grade commercially available.

2.3. Preparation of insect homogenates

All preparations were carried out at 4 °C. One gram of 5th instar housefly was separately homogenized in a glass homogenizer with 15 ml 100 mM phosphate buffer, pH 7.4. The resultant homogenates were filtered through four layers of cheesecloth. The homogenates were centrifuged at 12,100 × *g* at 4 °C for 20 min using an Eppendorf Centrifuge 5417R. The supernatants were reserved as crude enzyme extracts. For the determination of 17α- or β-hydroxysteroid dehydrogenase activity, the crude enzymic extracts were transferred to a 15 ml polycarbonate ultracentrifuge tube and centrifuged at 141,000 × *g* at 4 °C for 1 h in a Beckman L8-M ultracentrifuge using a Ti 50 rotor. The supernatant was reserved as cytosolic fraction and the pellet rinsed twice with 2 ml of resuspension buffer, 200 mM phosphate buffer (pH 7.5) containing 20% (v/v) glycerol and 1 mM EDTA. Then the pellet was resuspended in this buffer using 2 ml glass homogenizer and made a volume of 2 ml with the resuspension buffer to give the microsomal fraction.

2.4. Aflatoxin B_1 (AFB_1) metabolism in both permethrin-susceptible (aabys) and -resistant (ALHF) strains of housefly

Metabolism of AFB_1 was studied using an incubation mixture (250 μl final volume) consisting of 92 mM sodium phosphate buffer (pH 7.4), 0.5 mM NADPH, and 2 mg/ml protein of the crude extract preparations of insects. After a pre-incubation period of 10 min at 37 °C, respective AFB_1 was added as substrate to the reaction mixture. After 1 h, incubation reactions were stopped by adding 1 ml ice-cold methanol containing AFG_1 (10 μM) as an internal standard. This mixture was centrifuged at 12,100 × *g* for 10 min at room temperature. The supernatant was analyzed by reverse-phase HPLC on a Supercosil LC-18 column (250 × 4.6 mm) equipped with a fluorescence detector; excitation 365 nm, emission 455 nm (Lee and Campbell, 2000). The mobile phase was water:acetonitrile:methanol (60:20:20). On this system, retention time for AFB_1, AFB_2, AFG_1, AFB_{2a}, AFM_1, $AFLM_1$, and AFBO-GSH conjugate were 13.6, 12.1, 10.1, 7.7, 7.3, 6.5, and 6.1 min, respectively. A series of measured amounts of each aflatoxin metabolite was used to calibrate standard curves for quantitation of biotransformation products by HPLC peak area integration.

2.5. Determination of L-DOPA, phenylalanine, and tyrosine contents

All preparations were carried out at 4 °C. For the estimation of the range to detect L-DOPA, phenylalanine, and tyrosine amount in housefly larvae, 1 g of larvae was homogenized in a glass homogenizer with 10 ml of 100 mM phosphate buffer, pH 7.4. The resultant homogenates were filtered through four layers of cheesecloth. The homogenates (0.5 ml) were immediately mixed with 50% H_3PO_4 solution (0.5 ml). This mixture was centrifuged at 12,100 × *g* for 10 min at room temperature. The supernatant was analyzed by reverse-phase Supelcosil LC-18 column (250 × 4.6 mm) equipped with a fluorescence detector, with excitation at 281 nm and emission at 314 nm for L-DOPA and tyrosine, and with excitation at 215 nm and emission at 283 nm for tyrosine and phenylalanine as described previously (Steiner et al., 1996). The isocratic elution systems (1 ml/min) used were 1.7% aqueous H_3PO_4: 80% aqueous acetonitrile (98:2, v/v). L-DOPA has a retention time of 8.6 min, while tyrosine and phenylalanine has a retention time of 14.1 and 29.3 min, respectively. A standard curve was set up for quantitative analysis by integration of peaks of a series of each standard.

2.6. Tyrosinase activity

The assay was performed as previously described (Kubo et al., 1995). An aliquot (1 ml) of 2.5 mM L-DOPA was mixed with 1.9 ml of 0.1 M phosphate buffer (pH 6.8), and incubated at 25 °C for 10 min. Then, 0.1 ml of the crude enzyme extracts was added to the mixture to immediately measure the initial rate of linear increase in optical density at 495 nm, based on the dopachrome.

2.7. 17α- or β-hydroxysteroid dehydrogenase (17α- or β-HSD) activity

Metabolism of estrone was studied using an incubation mixture (610 μl final volume) consisting of 92 mM sodium phosphate buffer (pH 7.4), 0.2 mM NADPH, and 2 mg/ml protein of the crude extract of housefly larvae. After a pre-incubation period of 10 min at 37 °C estrone was added as substrate to the reaction mixture. After 1 h incubation reactions were stopped by adding 1 ml ice-cold methanol. This mixture was centrifuged at 12,100 × *g* for 10 min at room temperature. The supernatant was analyzed by reverse-phase Supelcosil LC-18 column (250 × 4.6 mm) equipped with a fluorescence detector, with excitation at 280 nm and emission at 312 nm as described previously. Mobile phase was a mixture of water:acetonitrile (58:42, v/v). β-estradiol has a retention time of 10.1 min, while 17α-estradiol and estrone has a retention time of 11.9 and 15.2 min, respectively. A standard curve was set up for quantitative analysis by integration of peaks of a series of each standard estrogens. For inhibition studies of pyrethroid insecticides on 17α- and β-HSD activities, bifenthrin, cypermethrin, deltamethrin, and permethrin (10 mM in final concentration) were mixed with the reaction mixture as described as previously. The inhibition of pyrethroids on 17α- and β-HSD activities were calculated as remaining activity (%) compared with control.

2.8. Phenylalanine-4-hydroxylase activity

All preparations were carried out at 4 °C. One gram of housefly larvae was homogenized in a glass homogenizer

with 10 ml, 100 mM phosphate buffer, pH 7.4, containing 1 mM EDTA, 0.1 mM DTT, and 0.4 mM PMSF. The resultant homogenates were filtered through four layers of cheesecloth. The homogenates were centrifuged at 12,100 × *g* at 4 °C for 20 min using an Eppendorf Centrifuge 5417R. The reaction mixture was consisted of 0.25 ml crude enzyme extracts, 0.25 mL 100 mM phosphate buffer, pH 7.4, and 20 μl of phenylalanine (100 mM). The hydroxylation reaction was initiated by addition of 0.1 ml of NADPH (10 mM). Control was without the addition of NADPH in the reaction mixture. The reaction was stopped by addition of 50% H_3PO_4 solution (0.5 ml). This mixture was centrifuged at 12,100 × *g* for 10 min at room temperature. The supernatant (20 μl) was analyzed for remaining phenylalanine content as above. The enzyme activity was calculated as picomoles of phenylalanine disappeared per minute per mg protein.

2.9. Determination of protein content

Protein was determined by the method of Bradford (1976) using 50 μl of sample solution and 2.5 ml of diluted (1–5) Bio-Rad Coomasie Blue concentrate.

2.10. Statistical analysis

Means of five replicates were compared and tested for significant difference by Student *t*-test at the $P = 0.05$ level (SAS, 1995).

3. Results

AFB_1 assays of ALHF strain of housefly larvae showed three major biotransformation products: AFB_{2a}, AFM_1, and AFL (Table 1). These chief products were also found in aabys strain. AFL production rate of ALHF larvae was 5-fold lower than that of aabys. Differences between ALHF larvae and aabys larvae in AFM_1 production are shown in Table 1. There are not different between ALHF and aabys for AFB_{2a} production.

Table 1
Aflatoxin B_1 metabolism in a permethrin-susceptible (aabys) and a permethrin-resistant (ALHF) strains of housefly larvae, *Musca domestica* L.

Metabolites[a]	aabys	ALHF
Aflatoxin M_1	0.60 ± 0.09 a[b]	0.33 ± 0.06 b
Aflatoxin B_{2a}	0.098 ± 0.01 a	0.086 ± 0.013 a
AFBO[c]	N.D. a	N.D. a
Aflatoxicol	42.0 ± 2.8 a	8.40 ± 0.76 b

N.D. denotes not detectable.

[a] The rate of metabolite production from aflatoxin B_1 was in nanomoles metabolite produced per minute per mg protein, except for AFBO.

[b] Means within a row followed by different letters are significantly different ($P < 0.05$).

[c] AFBO indicates aflatoxin B_1 8,9-epoxide.

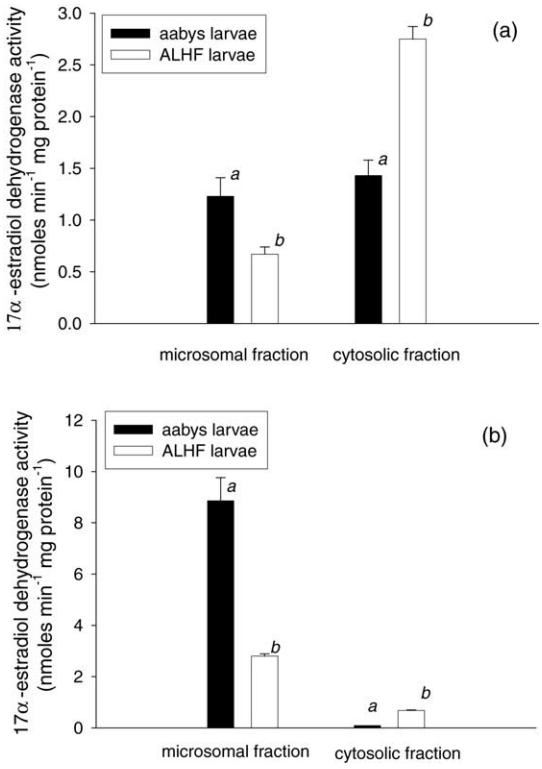

Fig. 2. 17α- and β-estradiol dehydrogenase activities of a permethrin-resistant (ALHF) and an insecticide-susceptible strain (aabys) of housefly larvae. The rate was expressed in nanomoles α- or β-estradiol produced per minute per mg protein. Means by different alphabetic words are significantly different ($P < 0.05$).

Two isomers of estradiol have been produced from estrone by both strains of housefly (Fig. 2). Production of larval cytosolic fraction in 17α-estradiol was found. On the other hand, about 2-fold lower 17α-estradiol production was found in larval microsomal fraction. The cytosolic fraction of ALHF larvae yielded 2-fold more 17α-estradiol than that of aabys larvae. In contrast, the microsomal fraction of ALHF larvae yielded 2-fold lower amount of 17α-estradiol than that of aabys larvae. Microsomal fractions from both strains yielded about 70-fold larger amounts of 17β-estradiol instead of 17α-estradiol in larvae than cytosolic fractions. The production rate of microsomal fraction of 17β-estradiol ALHF larvae yielded 3-fold lower than that of aabys larvae.

Inhibition studies on 17α- and β-HSD activity by pyrethroid insecticides showed that there was no inhibition of pyrethroid insecticides on the enzyme activities.

Phenylalanine-4-hydroxylase activity was determined (Table 2) and the production rate by ALHF was about 2-fold higher than that by aabys for larvae. DOPA content was determined and ALHF possessed 1.6-fold larger amounts of DOPA than aabys (Table 2). Phenylalanine and tyrosine amounts in both strains were not statistically different (data not shown). Tyrosinase activity was also determined and ALHF larval preparations showed 1.6-fold higher tyrosinase activity than aabys (Fig. 3).

Table 2
Phenylalanine-4-hydroxylase activity (PAH) and DOPA content of a permethrin-susceptible (aabys) and a permethrin-resistant (ALHF) strains of housefly larvae, *Musca domestica* L.

Type	aabys	ALHF
PAH[a]	0.24 ± 0.03 a[b]	0.51 ± 0.045 b
DOPA content[c]	0.28 ± 0.015 a	0.42 ± 0.03 b

[a] Phenylalanine-4-hydroxylase activity was in picomoles phenylalanine disappeared per minute per mg protein.
[b] Means within a row followed by different letters are significantly different ($P < 0.05$).
[c] DOPA content was in μg per gram housefly larvae.

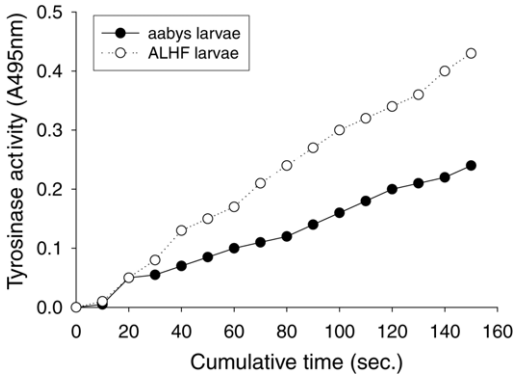

Fig. 3. Tyrosinase activities from enzyme extract of a permethrin-resistant (ALHF) and an insecticide-susceptible strain (aabys) of housefly larvae. The rate was calculated as absorbances at 495 nm.

4. Discussion

Mycotoxins are secondary metabolites produced by fungi that are toxic to mammals. Aflatoxins are a group of mycotoxins generally produced by *Aspergillus* and *Penicillium*. The mechanism through which aflatoxins are genotoxic appears to result from the formation of a single initial DNA adduct by an oxidized metabolite of AFBO. Biotransformation of aflatoxins can be linked interspecies variation (Lee and Campbell, 2000) and biotransformation products are distinctly different. Various cytochrome P450 species and dehydrogenases are involved in the biotransformation of AFB_1 (Lee and Campbell, 2000). Therefore, product profiles of AFB_1 biotransformation may indicate the types of enzymes involved and determine the changes of usual enzymic activity in an insecticide-resistant strain compared to the insecticide-susceptible strain. Table 1 shows that three major metabolites from AFB_1 are determined. AFM_1 production was 2-fold lower in the larvae of ALHF strain than those of aabys. For the AFB_{2a} production, there are no differences between the two strains. AFBO production was no statistical significant difference between two strains and aflatoxicol (AFL) production was significantly different. Therefore, a dehydrogenase activity converting AFB_1 to AFL was completely affected by the permethrin resistance in housefly.

On the other hand, one of insect dehydrogenase activities, 17α- or β-HSD activity converting estrone to 17α- or β-estradiol in both strains has been determined and ALHF strain possesses much lower levels of enzymic activities than aabys. Even though insects possess estradiol or other vertebrate steroid hormones, their roles are still in doubt (Swevers et al., 1991). In our findings, 17α- or β-HSD enzyme activities against estrone are significantly different between both strains. A series of inhibition kinetic studies on 17α- or β-HSD by pyrethroid insecticides indicates that there are no inhibitory effects of pyrethroids on the enzyme activities from both enzyme preparations. Therefore, there seems to be no changes of the enzyme structures, resulting in affinity changes of the enzyme to the substrates. A protein synthesis mechanism was differently controlled in ALHF after selection, giving a low level of protein synthesis of HSD in ALHF. However, this enzymic change is not essential for insect life. These results are very important because 17β-HSD is one of the enzymes catalyzing the last step in the ovarian estradiol production for human. Thus, enzymes with 17β-HSD activity catalyze the reactions between the low-active female sex steroid, estrone, and the more potent estradiol (Ghosh and Vihko, 2001). Therefore, long-term exposure of human to pyrethroid insecticides including permethrin may cause the changes on 17β-HSD activity. Pyrethroid insecticides are well known by their mimic effects on the 17β-estradiol receptors (Chen et al., 2002). It is first report that insecticide resistance to permethrin may suppress 17β-HSD synthesis.

Phenylalanine is an essential amino acid in insects and is a major substrate for phenylalanine-4-hydroxylase activity to produce tyrosine (tyr). We have determined phenylalanine, tyr, and L-(3,4-dihydroxyphenyl)alanine (DOPA) contents and phenylalanine-4-hydroxylase activities from the two different strains of housefly larvae. Phenylalanine and tyrosine content was not significantly different, but the phenylalanine-4-hydroxylase activity was significantly changed in ALHF strain. The phenylalnine-4-hydroxylase activity in ALHF strain was 2-fold higher than that in aabays strain. DOPA is formed in insects by enzymic tyrosine hydroxylation. The conversion of tyr to DOPA, which is initial and rate-limiting step in the biosynthesis of catecholamine neurotransmitters and in sclerosis of insects, demands a heme-containing mixed function oxidase (tyrosine 3-monooxygenase, EC 1.14.16.2; tyrosine hydroxylase). Melanin biosynthesis in insects starts similarly with the hydroxylation of tyr catalyzed by a copper-containing glycoprotein (tyrosinase, EC 1.14.18.1). In our findings, tyrosinase activity in ALHF has been increased, thus melanization rate may be increased in ALHF. These findings are previously reported (Fisher and Brady, 1980). Increased levels of phenylalanine-4-hydroxylase activity may be one example of changes of normal biosynthetic pathways in the permethrin-resistant housefly. In addition, an isozyme(s) which catalyzes phenylalanine to tyr may be also involved in the resistance mechanisms to pyrethroids in housefly such as the modulation of insecticide penetration into insect cuticles.

In summary many enzymic changes have been demonstrated in a permethrin-resistant strain of housefly compared to an insecticide-susceptible strain and these changes have

been resulted from the permethrin selection. Permethrin resistance in housefly can induce some protein levels to detoxify the toxic compounds and suppress other protein synthesis to balance for life. It may be one of evidence for evolution against insecticides in insects.

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References

Bourguet, D., Genissel, A., Raymond, M., 2000. Insecticide resistance and dominance levels. J. Econ. Entomol. 93, 1588–1595.

Bradford, M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72, 248–254.

Bull, D.L., Pryor, N.W., 1990. Characteristics of resistance in houseflies subjected to long-term concurrent selection with malathion and permethrin. Pest. Biochem. Physiol. 42, 211–226.

Castella, C., Castells-Brooke, N., Berge, J.B., Pauron, D., 1997. Expression and distribution of voltage-sensitive sodium channels in pyrethroid-susceptible and pyrethroid-resistant *Musca domestica*. Invert. Neurosci. 3, 41–47.

Chen, H., Xiao, J., Hu, G., Zhou, J., Xiao, H., Wang, X., 2002. Estrogenicity of organophosphorus and pyrethroid pesticides. J. Toxicol. Environ. Health A 65, 1419–1435.

Clark, J.M., Scott, J.G., Campos, F., Bloomquist, J.R., 1995. Resistance to avermectins: extent, mechanisms, and management implications. Annu. Rev. Entomol. 40, 1–30.

Fisher, C.W., Brady, U.E., 1980. Increased rate of melanization in hemolymph of American cockroaches (*Periplaneta americana*) and house crickets (*Acheta domesticus*) intoxicated by insecticides. Experentia 36, 93–94.

Ghosh, D., Vihko, P., 2001. Molecular mechanisms of estrogen recognition and 17-keto reduction by human 17beta-hydroxysteroid dehydrogenase 1. Chem. Biol. Interact. 130–132, 637–650.

Iyer, R.S., Harris, T.M., 1993. Preparation of aflatoxin B1 8,9-epoxide using *m*-chloroperbenzoic acid. Chem. Res. Toxicol. 6, 313–316.

Kubo, I., Yokokawa, Y., Kinst-Hori, I., 1995. Tyrosinase inhibitors from Bolivian medicinal plants. J. Nat. Prod. 58, 739–743.

Lee, S.E., Campbell, B.C., 2000. *In vitro* metabolism of aflatoxin B1 by larvae of navel orangeworm, *Amyelois transitella* (Walker) (Insecta, Lepidoptera, Pyralidae) and codling moth, *Cydia pomonella* (L.) (Insecta, Lepidoptera, Tortricidae). Arch. Insect Biochem. Physiol. 45, 166–174.

Lee, S.E., Lees, E.M., 2001. Biochemical mechanisms of resistance in strains of *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) resistant to malathion and chlorpyrifos-methyl. J. Econ. Entomol. 94, 706–713.

Lee, S.E., Lees, E.M., Campbell, B.C., 2000. Purification and characterization of an esterase conferring resistance to fenitrothion in *Oryzaephilus surinamensis* (L.) (Insecta, Coleoptera, Silvanidae). J. Agric. Food Chem. 48, 4991–4996.

Li, J., Zhao, X., Christensen, B.M., 1994. Dopachrome conversion activity in *Aedes aegypti*: significance during melanotic encapsulation of parasites and cuticular tanning. Insect Biochem. Mol. Biol. 24, 1043–1049.

Little, E.J., McCaffery, A.R., Walker, C.H., Parker, T., 1989. Evidence of an enhanced metabolism of cypermethrin by a monooxygenase in a pyrethroid-resistant strain of the tobacco budworm (*Heliothis virescens* F.). Pest. Biochem. Physiol. 34, 58–68.

Liu, N., Scott, J.G., 1997. Inheritance of CYP6D1-mediated pyrethroid resistance in house fly (Diptera: Muscidae). J. Econ. Entomol. 90, 478–481.

Liu, N., Yue, X., 2000. Insecticide resistance and cross-resistance in the house fly (Diptera: Muscidae). J. Econ. Entomol. 93, 1269–1275.

Pepper, D.R., Osborne, M.P., 1993. Electrophysiological identification of site-insensitive mechanisms in knockdown-resistant strains (*kdr*, *super-kdr*) of the housefly larva (*Musca domestica*). Pest. Sci. 39, 279–286.

Raney, K.D., Meyer, D.J., Ketter, B., Harris, T.M., Guengerich, F.P., 1992. Glutathione conjugation of aflatoxin B1 exo- and endo-epoxides by rat and human glutathione *S*-transferases. Chem. Res. Toxicol. 5, 470–478.

SAS, 1995. SAS User's Guide: Statistics. SAS Institute, Cary, North Carolina.

Scott, J.G., Georghiou, G.P., 1986. The biochemical genetics of permethrin resistance in the Learn-PyR strain of house fly. Biochem. Genet. 24, 25–37.

Smith, T.J., Lee, S.H., Ingles, P.J., Knipple, D.C., Soderlund, D.M., 1997. The L1014F point mutation in the house fly Vssc1 sodium channel confers knockdown resistance to pyrethroids. Insect Biochem. Mol. Biol. 27, 807–812.

Sparks, T.C., Lockwood, J.A., Byford, R.L., Graves, J.B., Leonard, B.R., 1989. The role of behavior in insecticide resistance. Pest. Sci. 26, 383–399.

Steiner, U., Schliemann, W., Strack, D., 1996. Assay for tyrosine hydroxylation activity of tyrosinase from betlain-forming plants and cell cultures. Anal. Biochem. 238, 72–78.

Swevers, L., Lambert, J.G.D., De Loof, A., 1991. Synthesis and metabolism of vertebrate-type steroids by tissues of insects: a critical evaluation. Experentia 47, 687–698.

Ui-Tei, K., Nishihara, S., Sakuma, M., Matsuda, K., Miyake, T., Miyata, Y., 1994. Chemical analysis of neurotransmitter candidates in clonal cell lines from Drosophila central nervous system. I. ACh and L-dopa. Neurosci. Lett. 174, 85–88.

Walsh, S.B., Dolden, T.A., Moores, G.D., Kristensen, M., Lewis, T., Devonshire, A.L., Williamson, M.S., 2001. Identification and characterization of mutations in housefly (*Musca domestica*) acetylcholinesterase involved in insecticide resistance. Biochem. J. 359, 175–181.

Weill, M., Fort, P., Berthomieu, A., Dubois, M.P., Pasteur, N., Raymond, M., 2002. A novel acetylcholinesterase gene in mosquitoes codes for the insecticide target and is non-homologous to the ace gene Drosophila. Proc. Roy. Soc. Lond. B: Biol. Sci. 269, 2007–2016.

Williamson, M.S., Denholm, I., Bell, C.A., Devonshire, A.L., 1994. Knockdown resistance (*kdr*) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the housefly (*Musca domestica*). Mol. Gen. Genet. 240, 17–22.

Williamson, M.S., Denholm, I., Bell, C.A., Devonshire, A.L., 1993. Knockdown resistance (kdr) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the housefly (*Musca domestica*). Mol. Gen. Genet. 240, 17–22.

Zhao, Y., Park, Y., Adams, M.E., 2000. Functional and evolutionary consequences of pyrethroid resistance mutations in S6 transmembrane segments of a voltage-gated sodium channel. Biochem. Biophys. Res. Commun. 278, 516–521.